$^{14}C,^2H_1,^3H_1$]acetate by this route gave methionine containing a methyl group which, if chiral, must have R configuration and R-[2-$^{14}C,^2H_1,^3H_1$]acetate gave (*methyl-S*)-[*methyl-*$^{14}C,^2H_1,^3H_1$]methionine. The overall yields in these syntheses were about 10% and the T/^{14}C ratios of starting material (e.g., 7.01 and 9.91) and product (e.g., 7.04 and 9.43) were essentially unchanged.

Previous work from this laboratory[12] has shown that *Streptomyces griseus* (ATCC 12648) incorporates the methyl group of methionine into the *C*-methyl and *N*-methyl groups of the antibiotic, indolmycin. This system was used to probe the steric course of a C-methylation reaction, the transfer of the methyl group of adenosylmethionine to the methylene carbon of indole 3-pyruvate.[13] Employing procedures described previously,[12] we incubated (*methyl-R*)- and (*methyl-S*)-[*methyl-*$^{14}C,^2H_1,^3H_1$]methionine (T/^{14}C 7.04 and 7.08, respectively) with shake cultures of *S. griseus* and isolated the resulting indolmycin (T/^{14}C 7.12 and 6.94). Following removal of the *N*-methyl group by alkaline hydrolysis, the indolmycin was further degraded by Kuhn–Roth oxidation to give acetic acid from the *C*-methyl group[12] (T/^{14}C 6.47 and 6.99, respectively). The chirality of the two specimens of acetate was determined as indicated above (39 and 63% tritium retention in the fumarase reaction, respectively) and the results were compared to those obtained in the chirality analysis of the original acetate samples used for the synthesis of the methionines (31 and 71% tritium retention in the fumarase reaction, respectively).

These results show that the above synthesis of methionine did produce material containing a chiral methyl group. The acetate obtained from the degradation of indolmycin had the same configuration as the acetate used for the synthesis of the respective methionine. Since the synthesis of methionine from acetate involves one inversion of configuration, it follows that the C-methylation of indole 3-pyruvate proceeds with net inversion of configuration at the methyl group.[14] This result complements our earlier finding[15] of retention of configuration at the methylene carbon of indole 3-pyruvate in this reaction and allows us to describe the stereochemistry of the process as shown in Scheme II. The somewhat (40%) lower stereochemical purity of the methyl group in the product compared to the starting acetic acid would indicate that some racemization takes place during one of the chemical steps, e.g., the alkylation of homocysteine or the Kuhn–Roth oxidation or, less likely, that the enzymatic methylation reaction is not entirely stereospecific.

Methyl transferase reactions are believed to proceed by nucleophilic attack on the methyl group of *S*-adenosylmethionine[16] and Hegazi et al.supc17 have concluded on the basis of secondary isotope effect measurements that catechol-*O*-methyl transferase involves an S_N2-like transition state. Inversion of configuration at the methyl group, as observed in this study, indicates that an odd number of nucleophilic displacements takes place and thus suggests that the methyl group is transferred directly from the donor to the acceptor without generation of a methylated-enzyme intermediate.

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Application of a Novel Thermistor Mercury Electrode to the Study of Changes of Activity of an Adsorbed Enzyme on Electrochemical Reduction and Oxidation

Sir:

The strong adsorption of the amino acid cystine and proteins (e.g., albumin and insulin) from aqueous solutions onto a mercury electrode surface, presumably through interaction of the disulfide bond with mercury, has been reported.[1-3] The question of whether enzymes adsorbed on mercury maintain their activity, how this activity is changed upon electroreduction, and the application of electrodes based on such adsorption for analytical purposes is the subject of this communication. It is known that enzymes covalently bound to surfaces or immobilized in gels can be employed as catalysts,[4,5] but that metal ions, such as Hg^{2+}, often deactivate enzymes. The activity of the adsorbed enzyme was determined in this study by measuring temperature changes resulting from the action of the enzyme urease on a substrate (urea) solution; similar techniques have previously been employed in studies involving dissolved enzymes.[6]

The thermistor mercury electrode (tme) was constructed by sealing a Veeco Engineering Co. 32A223 thermistor in a U-shaped glass tube with epoxy cement. A small drop of mercury (0.1 ml) covers the thermistor and serves as both the adsorption surface for the enzyme and to conduct heat from the electrode surface to the thermistor. Electrical contact to the mercury is made with a fine Pt wire. Electrodes containing thermistors in close proximity to solid metals have previously been described for measurement of heats of electrode reactions.[7] Temperature changes, determined using a dc Wheatstone bridge arrangement, followed previous practice.[6] The thermostat bath at 25 °C was maintained constant to within 0.001 °C.

When urease is adsorbed on a tme by immersion for several minutes in a solution of 4×10^{-5} M urease, then washed and immersed in a pH 7.3 phosphate or Tris buffer alone and subjected to cyclic voltammetry, the characteristic reduction and oxidation peaks of an adsorbed material (similar to those found with cystine, insulin, and albumin[1-3]) are observed (Figure 1a). The peak currents are directly proportional to scan rate, v; Epc = −0.58 V vs. sce and Epa = −0.52 V vs. sce. The

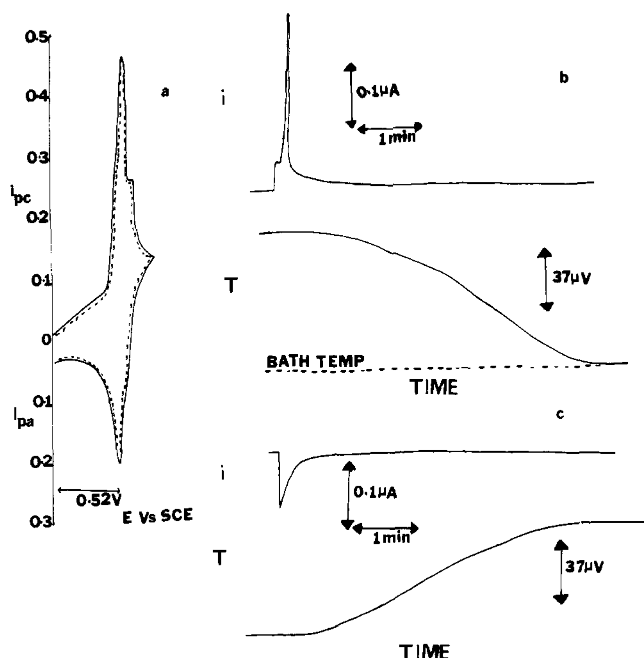

Figure 1. (a) Cyclic voltammogram of adsorbed urease in 0.01 M phosphate buffer at thermistor mercury electrode. v = 45 mV/s. Electrode after use in urea solution (- - -). (b) Upper curve: current where a tme containing adsorbed urease is stepped to −0.58 V in 10 mM urea in Tris (0.01 M) buffer, pH 7.3. Lower curve: thermistor response during and following the reduction process. (c) Upper curve: current when tme with adsorbed urease is stepped to −0.40 V (following reduction at −0.58 V). Lower curve: thermistor response during and following the oxidation process.

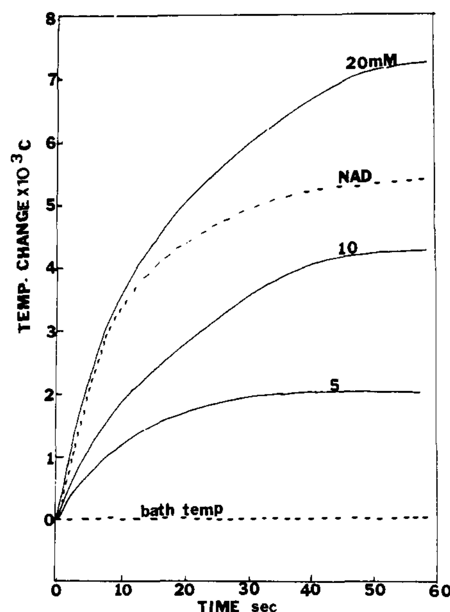

Figure 2. Change of temperature of thermistor mercury electrode containing adsorbed enzyme: solid lines, adsorbed urease with pH 7.3 buffer solution containing different amounts of urea; dotted line, adsorbed alcohol dehydrogenase in solution containing 0.10 M ethanol and 1.2 mM NAD^+. Zero time in all curves taken where initial temperature rise crosses the bath temperature.

temperature changes occurring at the tme during the reduction and oxidation processes are measurable but very small. When the tme, prepared by equilibration with enzyme solution and washing is immersed in a pH 7.3 buffer solution containing urea, in a thermostat bath, the temperature, after a small drop upon transfer, increases and finally levels off at a steady state value governed by the rate of the hydrolysis reaction, the mass transfer of urea to the electrode, and the thermal conduction of heat by the solution and electrode material (Figure 2). The rate of temperature rise and the final steady state value increases with increasing urea concentration. For 0.5 M urea, however, much smaller temperature changes (ca. 0.87×10^{-3} °C) were found. At this concentration substrate inhibition[9] probably reduces the reaction rate and thus the steady state temperature. When mercuric nitrate is added to the urea solution at a level of 10^{-4} M, much smaller steady state temperatures are observed, indicating that the temperature rise originally seen can be attributed to the enzyme catalyzed hydrolysis, which is inhibited by mercury(II). The heat of reaction for the urease-catalyzed hydrolysis of urea at pH 7.0 is about −14.7 kcal/mol in phosphate buffer. Very similar temperature changes are observed when the tme is treated with an alcohol dehydrogenase solution, then washed and immersed in a pH 11.9 solution containing ethyl alcohol and nicotinamide adenine dinucleotide (NAD^+) (Figure 2).

A simple visual demonstration of the action of urease adsorbed on mercury can be obtained in the following manner. An amalgamated copper foil is dipped into a urease solution for several minutes then washed well with water and immersed in an unbuffered solution of urea containing a few drops of bromcresol-purple indicator. A purple color forms around the foil signaling the production of ammonia from the adsorbed enzyme catalyzed hydrolysis.

The temperature changes which occur when the tme with adsorbed urease immersed in the urea solution is subjected to electrochemical reduction and oxidation are also informative. A tme with adsorbed urease was immersed in a 10 mM urea solution of pH 7.3. After the steady state temperature due to heating by the hydrolysis reaction was attained, the potential was swept to −0.58 V and held at this value. After this reduction the temperature dropped to the bath temperature, signaling destruction of enzyme activity (Figure 1b). The relatively slow decrease in temperature (compared to the time required for the electroreduction process) probably reflects the slow response of the thermistor and the time required to dissipate the heat of the mercury and glass of the tme and the surrounding solution. Injection of additional urea to increase the concentration by 5mM did not change the temperature; a similar injection at an unreduced urease-tme brings about an immediate temperature rise.

If following reduction the potential of the tme was scanned to −0.40 V and held at this value, the temperature rose again, leveling after about 10 min to a steady-state value about 78% of that obtained with the original tme (Figure 1c). This behavior indicates that enzyme activity is at least partially restored upon reoxidation. Reduction at −0.58 V following the oxidation scan again caused the temperature to return to the bath value. Similarly when an electrode which had been subjected to the reduction-oxidation cycle is immersed first in water held in a thermostat (to allow it to come to the temperature of the bath) and then transferred into a 5 mM urea solution (pH 7.3), the temperature rises finally attaining a steady state value 1.9×10^{-3} °C above the bath temperature, just as observed with a tme with adsorbed urease which had not undergone electrochemical experiments. Trials with solutions containing higher concentrations of urea showed larger temperature changes. Upon removal from the urea solution, the tme showed cyclic voltammetric behavior virtually identical with that observed before the hydrolysis experiment (Figure 1a).

The results demonstrate that enzymes adsorbed at a mercury surface remain active, so that the active site of the enzyme is not significantly modified by the process. There is much current interest in chemically modified electrode surfaces,[10-13] and the results presented here suggest that the strong adsorption of enzymes is a particularly simple method of modifying a mercury electrode. These electrodes can be used for

analytical purposes and also to probe the conformation and configuration of adsorbed molecules and enzymes. The application of enzymes which catalyze reactions to form electroactive products, so that they behave as heterogeneous electrocatalysts, is particularly of interest. The changes in behavior of the adsorbed urease can be attributed to changes in conformation upon reduction and reoxidation. On reduction the disulfide bond oriented at the mercury surface is broken (forming -SH or $-S^-$) and the free movement at this site is sufficient to alter the distant active site and decrease the activity. The sulfhydryl groups do not move far apart, however, since reoxidation causes restoration of at least some of these bonds and thus enzymatic activity. Analogous behavior has been seen previously for enzyme systems. Thus the reversible denaturation of proteins is well-known and it has been shown that proteins (e.g., ribonuclease) containing disulfide bonds which have been ruptured by reductive cleavage in denaturing solvents will refold almost quantitatively upon oxidation to the native state.[14] Similarly the large change in the activity of a bound enzyme upon a small structural perturbation of a distant site has precedence in the work of Berezin et al.[15] These authors found that when trypsin was covalently bound to a nylon filament, the enzymatic activity was greatly decreased upon mechanical extension of the filament. Indeed these authors believe that deformations of the native conformation as small as 0.5 Å are sufficient to cause changes in the catalytic activity. The possibility of varying enzyme behavior by electron transfer at a surface adds an additional degree of control (and also added constraints) to modified electrodes with adsorbed enzymes. The possibility that such control processes are utilized in biological systems bears further investigation.[16]

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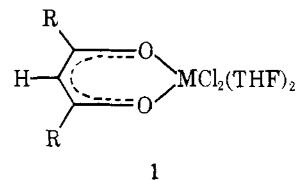
Paramagnetic Organometallic and Dialkylamide Complexes of Trivalent Titanium, Vanadium, and Chromium Stabilized by Diketonate Ligands

Sir:

Organometallic complexes of the early transition metals[1] and, in particular, paramagnetic complexes are generally very reactive and unstable. Large, bulky ligands such as $-C(CH_3)_3$, $-CH_2Si(CH_3)_3$, and $-N[Si(CH_3)_3]_2$, however, have yielded some very interesting and stable complexes. We are currently interested in preparing organometallic complexes of the early metals[2,3] and sought new starting materials for these reactions. The most commonly used reagents are the insoluble polymeric anhydrous metal halides and their tetrahydrofuran derivatives, i.e., $TiCl_3(THF)_3$, but these materials often lead to undesirable products.[4] We have now discovered a new series of compounds of trivalent titanium, vanadium, and chromium **1**, that are useful reagents for the syntheses of a variety of new and unusual paramagnetic, organometallic compounds.



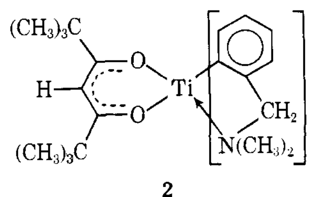
1

M = Ti, V, Cr
R = $-CH_3$, $-C_6H_5$, $-C(CH_3)_3$

The titanium and chromium derivatives are prepared directly from $MCl_3(THF)_3$ and 1 equiv of the appropriate 1,3-diketone in tetrahydrofuran. For example, a suspension of anhydrous $TiCl_3$ (35.0 g) in 500 ml of dry THF was refluxed under nitrogen for 2 h, and dipivalolylmethane (DPM-H) (41.8 g) was added dropwise. The solution was stirred magnetically for 1 h, cooled, and filtered and the solvent was removed to give red-purple crystals that were washed from the flask with pentane and dried. The yield of $(DPM)TiCl_2(THF)_2$ was 97.0 g (96%). The analogous vanadium complexes were prepared in only low yield by this procedure; however, good yields may be obtained by the addition of a THF solution of the sodium or potassium salt of the diketone (prepared in situ) to a refluxing solution of $VCl_3(THF)_3$ in tetrahydrofuran. The diketonate complexes $(diket)VCl_2(THF)_2$ were isolated as brown or yellow-green air-sensitive crystalline solids.

The magnetic moments of **1** were measured in solution by the Evans method[5] and were in the range 1.7–1.9, 2.6–2.7, and 3.7–3.9 μ_B for the titanium, vanadium, and chromium complexes, respectively. These values are in the range expected for octahedral d^1, d^2, and d^3 electronic configurations[6] of trivalent Ti, V, and Cr.

The addition of 2 equiv of Li-o-$C_6H_4CH_2N(CH_3)_2$ to a solution of $(DPM)TiCl_2(THF)_2$ in ether under an inert atmosphere at room temperature gave a dark green solution containing a suspension of lithium chloride. From this solution **2** was isolated in ~70% yield as a dark green, very air-sensitive



2

solid. It decomposes over a period of several days when stored under nitrogen at room temperature but may be stored for months at −40 °C without any apparent decomposition. Its electronic spectrum is typical of octahedral d^1 complexes[7] with an absorption band at 385 nm and a shoulder at 656 nm. The magnetic moment of 1.7 μ_B is also consistent with trivalent titanium. Hydrolysis of a benzene solution of **2** with D_2O gave $C_6H_4DCH_2N(CH_3)_2$, confirming the metalation of the benzyldimethylamine ligand. The vanadium analogue of **2** was synthesized in an analogous fashion and isolated as a dark maroon, air-sensitive crystalline solid. Unlike the titanium complex it may be stored under nitrogen at room temperature without decomposition. The magnetic moment (2.7 μ_B) was close to the spin only value of 2.83 μ_B.